



## Comparison and Evaluation of CTGF and P2/MS as Noninvasive Markers in Fibrosis Evaluation in Chronic Liver Diseases

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### Research Article

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### ABSTRACT

**Background/Aims:** To compare CTGF (connective tissue growth factor) and P2/MS [platelet count (109 / L)] 2 / [monocyte fraction (%) x segment neutrophil fraction (%)] ratio as non-invasive tests with liver biopsy in the detection of liver fibrosis. Thus, to determine whether CTGF and P2/MS index results can be used as a noninvasive marker, in the diagnosis of chronic liver disease

**Materials and Methods:** A total of 52 chronic hepatitis patients with concurrent liver biopsy (27 female, 25 male) were included in the study group. The subjects' ages ranged between 18 and 70, and none of the subjects had undergone liver disease-related treatment. The control group was made up of a total of 31 healthy individuals without liver disease (18 female, 13 male) from similar age groups. Liver biopsy results of patients were reported at 7 stages according to Modified Knodell Scoring (ISHAK).

**Results:** CTGF levels were higher in patients with chronic hepatitis (1.06±0.70 ng/ml) than the control group (0,72±0,32 ng/ml) (P<0,05). When the P2/MS indexes were compared according to the liver biopsy results of the patients in the study group, the difference was found to be significant. As the degree of fibrosis increased, P2/MS index decreased.

**Conclusions:** As a noninvasive test, it was seen that CTGF and P2/MS is parallel to liver biopsy in reflecting liver fibrosis. These noninvasive markers can be used where liver biopsies cannot be performed or where repetition is necessary, and in the follow-up of patients for early detection of fibrosis.

**Keywords:** Fibrosis, Liver, CTGF

## Kronik Karaciğer Hastalıklarında Fibrozisin Değerlendirilmesinde CTGF VE P2/MS' nin Noninvaziv Belirteçler Olarak Yerinin Araştırılması

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### Öz

**Amaç:** Bu çalışmanın amacı karaciğer fibrozisinin saptanmasında non invaziv yöntemler olarak CTGF (Konnektif doku büyüme faktörü) ve P2/MS (fragmente monosit (%) x nötrofil fraksiyonu (%)) yi araştırmak ve karşılaştırmak

**Materyal-Metot:** Çalışma grubuna 52 tane eş zamanlı karaciğer biyopsisine sahip kronik hepatit tanısı olan hasta (18-70 yaş aralığında daha önce karaciğere yönelik tedavi almamış olan 25 kadın, 25 erkek) dahil edildi. Karaciğer biyopsi sonuçları Modifiye Knodell skorlamasına göre 7 evreye ayrıldı. Kontrol grubu karaciğer hastalığı olmayan 31 sağlıklı bireyden ( 18 kadın, 13 erkek) oluşturuldu.

**Sonuçlar:** CTGF seviyeleri kronik hepatitli hastalarda (1.06±0.70 ng/ml), kontrol grubuna (0,72±0,32 ng/ml) göre daha yüksek bulundu (p<0,05). Fibrozis evresi arttıkça P2/MS indeksi azaldı. Noninvaziv testler olarak CTGF ve P2/MS nin karaciğer fibrozisini yansıtmada anlamlı olarak değiştiği tespit edildi. Bu noninvaziv testler karaciğer biyopsilerinin yapılamadığı ve tekrarının gerekli olduğu durumlarda fibrozisin erken saptanmasında hastaların takibinde önemli yer tutabilir.

**Anahtar sözcükler:** CTGF, P2/MS, Fibrozis

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## Introduction

Chronic liver diseases (CLD) are major causes of morbidity and mortality worldwide. Cirrhosis is the result of chronic inflammation and development of fibrosis that leads to various complications of CLD. Histological findings of liver fibrosis include point hepatocellular necrosis, chronic inflammatory cell infiltration in portal areas, damage caused by parenchymal and non-parenchymal cells, cytokines and other extracellular stimulants, and the response to this injury. Myofibroblasts are main effectors of fibrosis in all tissues and make a major contribution to other aspects of the wound healing response, including regeneration and angiogenesis. The prognosis and clinical curability largely depend on the extent of liver fibrosis. So degree of liver damage is valuable in determining the prognosis and diagnosing cirrhosis. Cirrhosis is the eleventh most common cause of death globally, accounting for an estimated 2 million deaths per year, indicating that cirrhosis deaths have risen from 899.000 to more than 1.32 million from 1990 to 2017<sup>1</sup>. The absolute burden of viral hepatitis has also increased, although the availability of effective vaccines and treatments may reduce the burden of these diseases in the years to come. Early diagnosis of CLD with noninvasive methods enables initiation of specific measures or treatments to prevent disease progression and improve survival. CTGF is a member of the Cellular Communication Network gene family, which contains the CTGF itself, *cyr 61*, *nov*, *eIM1*, *Cop1*, and *WISP-3*<sup>2</sup>. It is composed of four modular domains: insulin-like growth factor-binding protein (IGFBP), von Willebrand factor type C repeat, thrombospondin type 1 repeat, and cysteine knot-containing carboxyl domain. These domains perform various functions by acting on different factors including cell-surface receptors, cytokines and extracellular matrix proteins<sup>3</sup>. CTGF plays an important role through these various actions in important physiological and pathophysiological processes; such as embryogenesis, implantation, angiogenesis, chondrogenesis, tumorigenesis, differentiation, wound healing and fibrosis<sup>4</sup>.

CTGF is commonly up-regulated in the liver fibrosis<sup>5</sup>. In addition; CTGF is an important fibrosis-promoting mediator downstream target of TGF- $\beta$  that plays a key role in the liver fibrosis<sup>6,7</sup>. Also, due to its downstream localization in different pathways leading to fibrosis, CTGF may be an attractive target for anti-fibrotic therapies

in liver fibrosis. Indeed, downregulation of CTGF expression with the help of siRNA has been demonstrated to reduce liver fibrosis in rodent models<sup>8</sup>.

P2/MS, a simple and noninvasive test that was developed and has been confirmed for the detection of hepatic fibrosis in Korean patients with virus-related CLD and nonalcoholic fatty liver disease<sup>9-12</sup>. P2/MS [platelet count (109 / L)] 2/ [monocyte fraction (%) x segment neutrophil fraction (%)] is evaluated as a noninvasive marker showing fibrosis of liver. In another method, two-dimensional shear wave elastography (2D-SWE), unlike the measurement made from a single point in liver biopsy, a large number of regions of interest (ROIs) are placed in the liver parenchyma, and measurements are made from multiple points; thus, a larger area of the liver can be evaluated. However, there is no consensus on objective criteria for confirming the reliability of measurements made in 2D-SWE techniques<sup>13,14</sup>. Needle biopsy is an important and necessary method in determining the diagnosis in chronic liver diseases, the degree of necroinflammatory activity, the fibrosis stage and verifying the diagnosis of cirrhosis. Although liver biopsy is a reliable method, it may cause serious complications. In a recent meta-analysis in 2021; incidence of major complications was 2.44%, with mortality at 0,01%, major bleeding at 0,48% and moderate/severe pain at 0,34%<sup>15</sup>. Due to these technical limitations and risks in needle biopsy, the search for a new approach to diagnosis and follow-up of chronic liver disease has emerged.

## Materials and Methods

A total of 52 chronic hepatitis patients with concurrent liver biopsy (27 female, 25 male) were included in the study group. The subjects' ages ranged between 18 and 70, and none of the subjects had undergone liver disease-related treatment. The control group was made up of a total 31 healthy individuals without liver disease (18 female, 13 male) from similar age groups. The subjects who had received any anti-viral agent prior to the biopsy, or those who had a co-infection (such as Hepatitis B, Hepatitis C, HIV, Hepatitis D) or any other chronic disease were excluded from the study.

The biopsy was conducted under ultrasonography with cutter needles with a trigger mechanism. Histological evaluation of the biopsy specimens was made according to the ISHAK scoring system: F0, no fibrosis (n=6);

F1, increased fibrosis in most portal areas (n=12); F3, portoportal bridging in most portal areas (n=7); F4, increased fibrosis in most portal areas together with bridging (n=2), F5, bridging with nodular formations (n=3); F6, cirrhosis (n=3). Majority of the patients fell into the 1/6 and 2/6 ISHAK stages (57.7%). Liver biopsy results of the patients were categorized into 4 groups. 0/6 no fibrosis, 1-2/6 low fibrosis, 3-4/6 moderate fibrosis, 5-6/6 advanced fibrosis.

Simultaneously with the liver biopsy, blood samples were withdrawn from the antecubital vein and examined in the appropriate tubes for the following items: HBsAg, anti-HBs, anti-HCV, HBeAg, anti-HBe, HBV-DNA, HCV-RNA, creatinine, ALT, AST, ALP, GGT, LDH, direct bilirubin, indirect bilirubin, amylase, albumin, total cholesterol, LDL cholesterol, triglyceride, alpha-fetoprotein, complete blood count (neutrophil function, monocyte function, platelet count), CTGF, pre-biopsy prothrombin time, and partial thromboplastin time.

$P2/MS \text{ index} = \frac{[\text{number of platelets } (10^9 / L)]^2}{[\text{monocyte fraction } (\%) \times \text{segmented neutrophil fraction } (\%)]}$ .

#### Statistical Analysis

The data were analyzed using the SPSS 22.0 program. The Mann-Whitney U test, the Kruskal-Wallis test and the Chi-square tests were applied because the parametric test assumptions could not be met in the evaluation of the data.

## Results

There is no difference between the study and control groups in terms of age and gender (25 male, 27 female; 13 male, 18 female; respectively). Of the 52 patients, 45 were diagnosed with chronic hepatitis B, 5 were diagnosed with chronic hepatitis C, and 2 had autoimmune hepatitis. CTGF, P2/MS, ALT, AST, GGT were compared between the patient and control group. CTGF, ALT, AST, GGT and total bilirubin levels were higher in the study group than in the control group. P2/MS scores were lower in the study group than control group and the CTGF levels were higher in patients with chronic hepatitis than the control group (Table 1).

When the P2/MS scores were compared, it was found to be significantly lower in the study group. The CTGF values of the groups according to fibrosis levels (no fibrosis, low fibrosis, moderate fibrosis, advanced fibrosis) were different, but this result was not statistically significant (Table2).

When the P2/MS scores were compared according to the liver biopsy results of the patients in the study group, the difference was found to be significant. Increasing fibrosis lead to a decreased P2/MS score (Table 3).

**Table I.** Comparison of the study and control groups in terms of the measured parameters

Parameters	Study	Control	p
CTGF (ng/ml)	1.06 ± 0.70	0.72 ± 0.32	0.007*
P2/MS	130.21 ± 72.87	225.03 ± 73.71	0.001*
ALT (U/L)	92.88 ± 109.92	19.09 ± 7.76	0.001*
AST/ALT	0.97 ± 0.65	1.33 ± 0.44	0.001*
AST (U/L)	68.42 ± 67.62	23.21 ± 7.67	0.001*
GGT (U/L)	57.13 ± 71.97	22.35 ± 11.06	0.011*
T.Bil(mg/dl)	1.02 ± 7.73	0.83 ± 0.32	0.008*

ALT: Alanine aminotransferase AST: Aspartate aminotransferase GGT: Gamma-Glutamyl Transferase T. Bil: Total Bilirubin

**Table II.** CTGF levels according to the degree of liver fibrosis.

Biopsy	CTGF (ng/ml)
No Fibrosis	0.65 ± 0.21
Low fibrosis	1.08 ± 0.68
Moderate fibrosis	1.40 ± 1.05
Advanced fibrosis	0.99 ± 0.29
Result	KW = 7.56 P= 0.056

**Table III.** P2/MS index according to the degree of liver fibrosis.

Biopsy	P2/MS $\bar{x} \pm s$
No fibrosis	186.42 $\pm$ 21.94
Low fibrosis	142.26 $\pm$ 65.78
Moderate fibrosis	103.33 $\pm$ 70.13
Advanced fibrosis	48.35 $\pm$ 38.46
Result	KW=14.38 P= 0.002*

## Discussion

Chronic liver disease is a major public threat and the second leading cause of loss of working life years<sup>16</sup>. The leading causes of liver mortality are rapidly changing due to achievements in the treatment of viral hepatitis. We need to detect fibrosis early in order to prevent the progression of fibrosis and for antiviral treatment<sup>17</sup>. Liver biopsy is important for diagnosis. It is usually well tolerated, but it has unavoidable risks. However, the characteristics of nonalcoholic steatohepatitis did not show good compatibility in the biopsy pairs and it was concluded that liver biopsy was not an ideal test<sup>18</sup>. Therefore, there is the need for a noninvasive parameter. Recent clinical trials have found that CTGF levels in fibrotic liver diseases have increased<sup>2-4</sup>. ROC curves indicate that the area under the curve in fibrosis, cirrhosis, and healthy volunteers is relatively 0.955, 0.887, the sensitivity (84-100%) and specificity (85-89%) of CTGF make it a potentially valuable marker for active fibrogenesis<sup>19-20</sup>. In a study of chronic hepatitis B, serum CTGF levels were found to be indicative of the severity of liver fibrosis<sup>21</sup>. Regardless of the etiology of the underlying disease, serum CTGF levels were seen as the strongest indicator of fibrosis and advanced disease states<sup>22</sup>.

In our study, we found that CTGF levels were higher in patients with chronic hepatitis compared to the control group. The CTGF values of the groups according to fibrosis levels (no fibrosis, low fibrosis, moderate fibrosis, advanced fibrosis) were different, but this result was not statistically significant. This is probably due to the difference in the number of patients in the fibrosis groups.

P2/MS, a noninvasive test, has been noted for its high diagnostic accuracy and low cost for chronic liver disease<sup>10</sup>. Thrombocytopenia is expected with fibrosis progression. Increased fibrosis and worsening portal hypertension lead to increased secretion and enlargement of the spleen, resulting in increased platelet destruction. In addition, fibrosis progression is associated with decreased thrombopoietin

production in hepatocytes, which reduces platelet production. Portal hypertension can also lead to sequestration of granulocytes and red blood cells like platelets, and a small number of granulocytes in circulation, respectively, may increase as a compensator with a serum GM-CSF (granulocyte monocyte stimulating factor) concentration. As a result, the ratio of neutrophils and monocytes may increase because serum GM-CSF concentration induces more production of neutrophils and monocytes than lymphocytes. In addition to the diagnostic value of P2/MS, there are some clinical advantages. From a practical point of view, it is easy and simple to calculate the P2/MS index in bedside patients and outpatients. From a technical point of view, this test does not need any standardization. Also due to its parameters, it can be obtained easily from complete blood counts. No extra cost or additional biochemical work is required.

One of the objectives of our study was to calculate the diagnostic accuracy of P2/MS index in different chronic liver disease etiologies and to determine the optimal thresholds for histological prediction of cirrhosis in patients and to compare P2/MS index results with CTGF, another noninvasive test. In our study, the P2/MS index of patients with chronic hepatitis was found to be lower than that of healthy volunteers. When the P2/MS scores were compared according to the liver biopsy results of the patients in the study group, the difference was found to be significant. When the values were compared in pairs, the difference between the non-fibrosis, low fibrosis, moderate fibrosis and advanced fibrosis was found to be insignificant. The fact that the patient group is not composed of a sufficient number of different etiologies may be considered as one of the limitations in our study. In conclusion, our study showed that as fibrosis grade increased, P2/MS score decreased.

In patients with chronic hepatitis B and C, the P2/MS index also appears to have the highest diagnostic accuracy for fibrosis, severe fibrosis,

and histological cirrhosis detection among noninvasive fibrosis scoring systems. This has led to the conclusion that P2/MS index can be used as a noninvasive fibrosis scoring system in patients with chronic liver diseases.

In our study, the difference in CTGF and P2/MS score in the study group was found to be significant, a result that we did not encounter in the literature review. The index of P2/MS was found to be higher in patients with low CTGF.

As a result; CTGF and P2/MS index as noninvasive tests were seen to be parallel to liver biopsy in reflecting liver fibrosis, but we found that P2/MS index is more valuable than CTGF in reflecting the degree of liver fibrosis. Non-invasive markers may be used for the early detection of fibrosis in cases where liver biopsies can not be performed or where repetition is necessary and in the follow-up of patients. In terms of an alternative to liver biopsy, there is a need for prospective studies with a sufficient number of patients from different etiologies. These results may provide supporting evidence for therapies targeting the TGF- $\beta$ 1 pathway and CTGF molecule to prevent and treat fibrosis in chronic liver diseases.

**Ethics committee Approval:** Ethics committee approval was received for this study from the Ethics Committee of the Cumhuriyet University (Date of Approval:15.02.2012; Reference number:2012-02/03)

**Informed Consent:** Written informed consent was obtained from patients who participated in this study

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**Author contributions:** Concept- S.Ç., H.A., A.A.; Design- S.Ç, S.Ç; supervision- O.Y., S.Ç., H.A.; resource- S.Ç.; materials-S.Ç., A.A.; Data collection and/ or procesing- H.A., S.Ç.; Analysis and/or interperation- S.Ç., Literature Search-S.Ç., H.A.; Writing- S.Ç., S.Ç., O.Y; Critical Reviews- O.Y., S.Ç., H.A.

**Conflict of interest:** No conflict of interest was declared by the authors.

**Data availability statement:** The laboratory data generated in this study is available from the corresponding author on reasonable request.

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**Abbreviations:** CTGF: Connective tissue growth factor, P2/MS:  $[\text{platelet count } (10^9 / \text{L})]^2 / [\text{monocyte fraction } (\%) \times \text{segment neutrophil fraction } (\%)]$ , ISHAK: Modified Knodell Scoring, GM-CSF: Granulocyte monocyte stimulating factor, HCC: Hepatocellular Carcinoma

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